Korean Journal of Life Science
Vol. 11. No. 3. 259~265, 2001

Protection of Mercury induced Acute Respiratory Injury by Inhaled Oxidizing Agent

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Abstract

Mercury vapor inhalation-induced acute respiratory failure (ARF) has been reported to be fatal. This study was designed to observe the possible mechanism of inhaled mercury vapor poisoning in the respiratory system. Sixty percent of rats (12/20) exposed to mercury vapor were dead within 72 hours of exposure whereas all the rats (20/20) exposed to mercury vapor combined with dithiothreitol (DTT) vapor survived. The histological observation showed that ARF was a direct cause of the death induced by mercury vapor inhalation, which was significantly circumvented by DTT vapor. Cyclic AMP mediated chloride secretion was inhibited by luminal side but not serosal side sulfhydryl blocking agents (Hg^{2+}, *p*-chloromercuribenzoic acid or *p*-chloromercuriphenyl sulfonic acid) in a dose-dependent manner in a primary cultured rat airway monolayer. The inhibitory component of cAMP induced chloride secretion was completely restored by luminal side DTT (0.5 mM). These results suggest that the oxidized form (Hg^{2+}) of mercury vapor (Hg0) contribute to ARF and subsequent death. This finding is important as it can provide important information regarding emergency manipulation of ARF patients suffering from by mercury vapor poisoning.

Key words – Mercury vapor, chloride secretion, airway epithelia, acute respiratory injury

Introduction

Mercury (Hg) is a toxic metal that is omnipresent in the environment. It have been reported that mercury affect on the immune system, renal system, oral and intestinal bacteria, reproductive system, and central nervous system through various animal and human experiments over the past several years[1-4]. However, effects to the respiratory system have not been well investigated even though there have been some case reports of acute respiratory failure (ARF) induced by mercury inhalation poisoning [5-12]. Although unusual in adults, cases of mercury vapor induced ARF in young children have been reported to be fatal. This study was designed to identify the possible mechanism of mercury vapor poisoning in the respiratory system.

The results show that the oxidized form (Hg^{2+}) converted from inhaled mercury (Hg0) affects the respiratory system. The poisoning effect of mercury vapor inhalation was significantly attenuated by accompanied inhalation of a reducing agent (dithiothreitol, DTT) in an *in vivo* experiment. Furthermore, the addition of sulfhydryl blocking agents into the luminal side strongly inhibited cAMP mediated choride secretion, and this inhibition was protected by DTT in the rat airway cultured model in the *in vitro* study. These results indicated that the oxidized form (Hga^{2+}) of mercury vapor (Hg0) is involved in the ARF induced by mercury vapor inhalation.

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MATERIALS AND METHODS

Exposure to dental mercury vapor in a rat model

Special cages were constructed for this experiment. Rats were fed (2 rats/cage) as usual except for putting a Petri dish (50 mm) containing dental mercury (99.999%, General Goldsmith Chemical & Metal Corp.) or two Petri dishes containing dental mercury and dithiothreitol (100 mM, Sigma), respectively.

Histological examination

After the rats were exposed to mercury vapor with or without reducing agent (DTT) for 72 hours, the surviving rats were killed. The lung, kidney and liver were isolated immediately for observations of histological changes. The isolated organs were fixed in 10% neutral buffered formalin and embedded with paraffin. Tissue slices were stained with routine hematoxylin-eosin.

Cell culture

The isolation and culture of rat airway epithelia was done as described previously [13-15]. Briefly, fresh excised tracheas were incubated at 4°C for 18-24 hours in a CaMg-free, serum-free modified Eagles minimum essential medium (MEM) containing 0.1% protease XIV (Sigma), and 50,000 U/ml penicillin and 50,000 μg/ml streptomycin (Gibco-BRL solution of both antibiotics). The cells were incubated in an atmosphere of 5% CO_2-95% air at 37°C in the LHC-8 medium containing 5% fetal bovine serum (Gibco-BRL) and 50,000 U/ml penicillin and 50,000 μg/ml streptomycin. Cells were cultured for 6-10 days before insertion into a modified, circulating Ussing chamber system constructed to accept SNAPWELL filters (World Precision Instrument, Sarasota, FL).

Measurement of short circuit current (Isc)

Transepithelial electrophysiologic measurements were performed in a modified Ussing chamber constructed to accept SNAPWELL filters. Cyclic AMP mediated chloride secretion (I_{sc}) was measured with a DVC-1000 voltage/current clamp (World Precision Instrument) with a voltage clamp mode. The bath solution in rat airway epithelial cells was a nominally bicarbonate-free Ringers solution that was composed of (in mM): 140 NaCl; 2.3 K_2HPO_4; 0.4 KH_2PO_4; 1.2 $CaCl_2$; 1.2 $MgCl_2$; 10 HEPES; and 10 glucose (pH 7.4).

Statistical methods

The results are expressed as means SE. Students tests were used for two group comparison, with $P<0.01$ considered significant.

RESULTS

The effect of mercury vapor inhalation on the respiratory system was observed in the rats fed in the specialized cages. Sixty percent of rats (12/20) exposed to mercury vapor in a cage (2 rats/cage) were dead within 72 hours of exposure whereas all the rats (20/20) exposed to dental mercury combined with DTT vapor survived. The general health of surviving rats after 72 hours exposure to mercury vapor for 72 hours was significantly bad compared to that of the rats exposed to mercury vapor combined with DTT, judged in terms of their mobility. The surviving rats of the two groups were killed. The isolated lung from the rats exposed to dental mercury vapor for 72 hours showed extensive lesions, including alveolar collapse with widening of interstitium, focal emphysematous changes and peribronchial thickening (Fig. 1, a2-1). Widened interstitium represents neutrophils and mononuclear cell infiltration and hemorrhage (Fig. 1, a2-2), but hyaline membrane formation was not observed. On the contrary, the lung isolated from rats exposed to dental mercury vapor combined with DTT vapor showed only mild interstitial congestion and hemorrhage (Fig. 1, b2). The liver isolated from rats exposed to dental mercury vapor for 72 hours did not show a significant change except for individual cell necrosis (Fig. 1, b2). The kidney

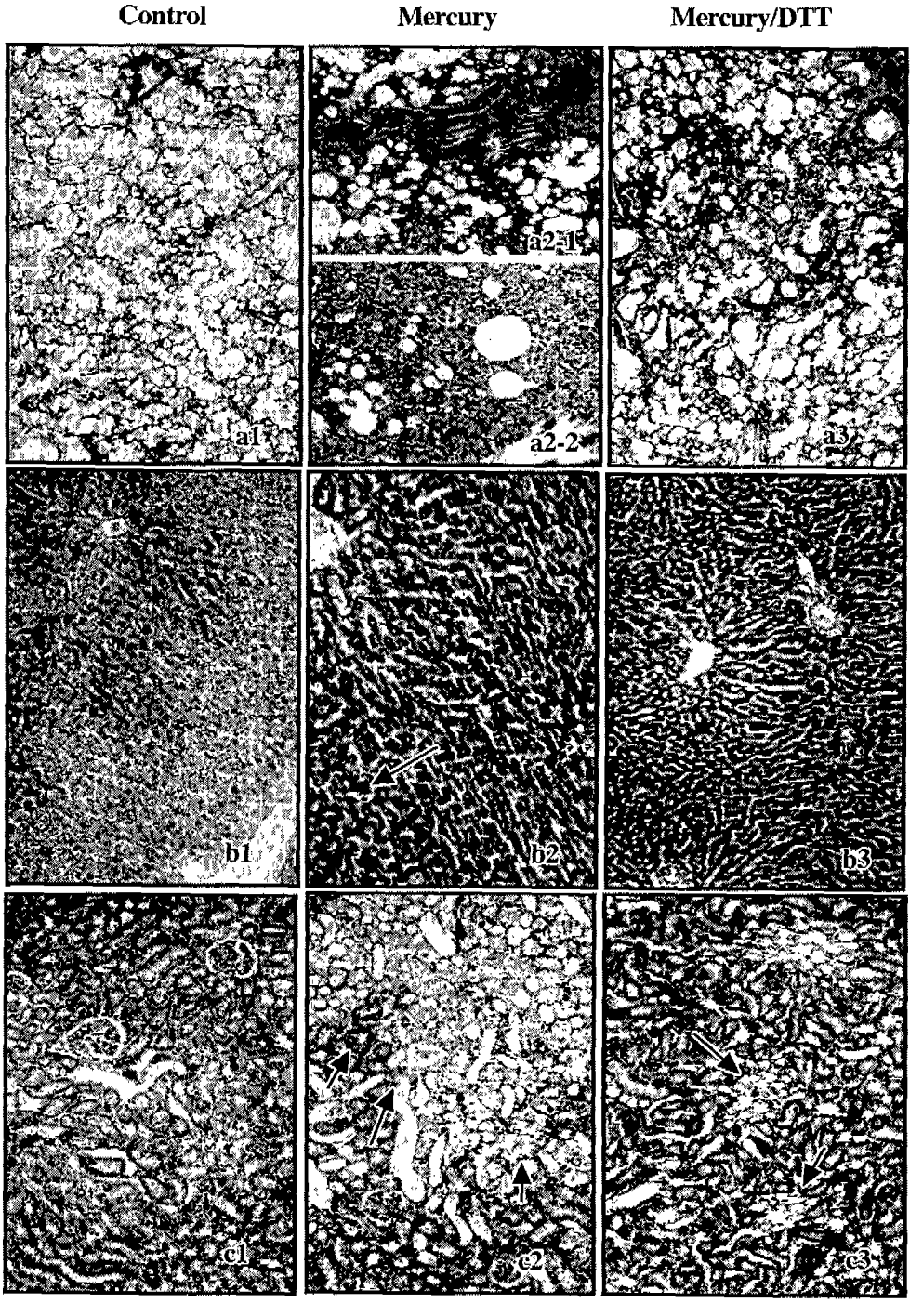

Fig. 1. The effect of mercury vapor inhalation (72 hours) with or without dithiothreitol (1 mM, DTT) vapor on the lung, liver and kidney of rat.

A. A special cage was constructed and 2 rats/cage were exposed to dental mercury vapor with (Rt.) or without (Lt.) DTT for 72hours.

B. Histologic findings of lung (a), liver (b) and kidney (c) of rat. a1, b1 and c1 (H-E, ×40) are from control, a2-1, b2, c2 (H-E, ×40) and a2-2 (H-E, ×100) are from rats exposed to dental mercury vapor, and a3, b3 and c3 (H-E, ×40) are from rats exposed to dental mercury and DTT (1 mM).

showed extensive acute tubular necrosis of proximal tubules in the dental mercury-exposed groups (Fig. 1 b2). The proximal tubule lining cells showed coagulation necrosis. The glomeruli were rather intact. The kidney from the dental mercury combined with DTT group showed atrophy of tubules, representing flattened shrinkage of lining cells and empty lumen, mimicking microcysts, focally (Fig. 1B c3).

The effect of Hg^{2+}, PCMB or PCMBS on cAMP mediated chloride secretion was observed in isolated rat airway epithelia *in vitro*. The luminal membrane of airway epithelia is the first defensive barrier against Hg0 vapor or Hg^{2+} in an *in vivo* situation. Figure 2 shows repre sentative current traces illustrating the inhibitory effect of HgCl2 (Hg^{2+}) after stimulation of Isc by forskolin which increases cytosolic cAMP. DTT, a reducing agent, completely restored the Hg^{2+} sensitive inhibitory component by > 90%. The application of HgCl2 (100 mM) into the serosal bath had no effect on Isc (data not shown) whereas as little as 10 nM apical Hg^{2+} inhibited cAMP mediated chloride secretion. The inhibitory effect of luminal side Hg^{2+} was dose-dependent. (IC50=3mM) (Fig. 2B). Other organic mercurial sulfhydryl blocking compounds such as PCMB and PCMBS also inhibited cAMP mediated chloride secretion in a dose-dependent manner (Fig. 2B). However, both serosal side and luminal side applications of other heavy metals such as $CdCl_2$ (0.5 mM), $ZnCl_2$ (0.5 mM) or $NiCl_2$ (0.5 mM) had no effect on cAMP mediated Isc (data not shown). The protective effect of DTT (1 mM) was observed to confirm the oxidizing effects of mercurial analogues. For this purpose, a maximum inhibitory concentration of mercurials ($HgCl_2$ = 1 mM, PCMB = 0.1 mM, PCMBS = 0.1 mM) shown in figure 3 was used. β-mercaptoethanol (β-ME), a reducing agent, also reversed the inhibitory effects of mercurials by > 90% (data not shown).

DTT or β-ME had no effect on the basal current and cAMP mediated chloride secretion without previous application of mercurials. These reducing agents increased chloride secretion only when cAMP mediated chloride

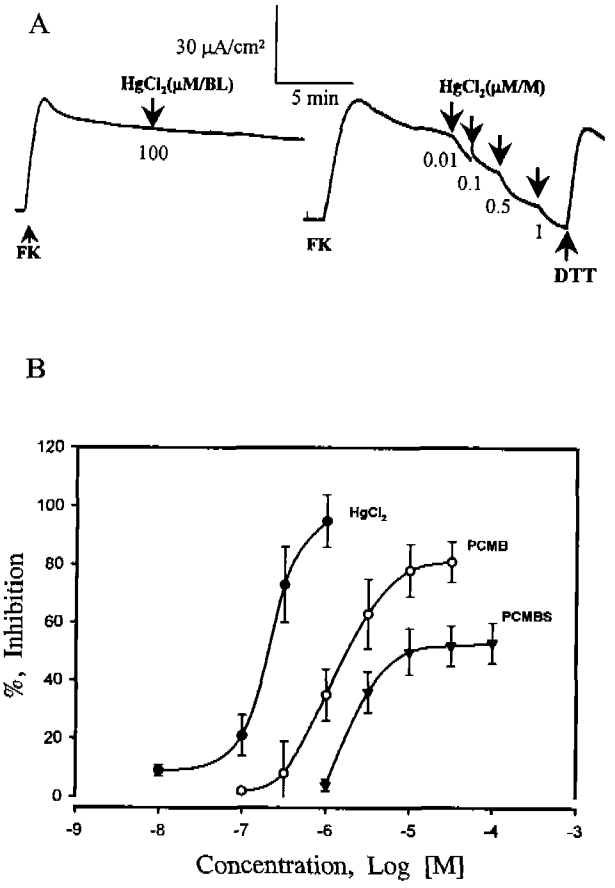

Fig. 2. The inhibitory effects of mercurials on the forskolin-induced short circuit current (Isc) in rat airway epithelial monolayer.
A-Lt.; $HgCl_2$ (0.1 mM) was added into serosal bath in the presence of forskolin (1 mM).
A-Rt.; $HgCl_2$ was added into luminal bath cumulatively in the presence of forskolin (1 mM). Dithiothreitol (DTT, 0.5 mM)) was added into luminal bath when forskolin-induced Isc was completely inhibited.
B. The dose-response curve of inhibition caused by SH blocking agents on the cAMP mediated Isc. PCMB; *p*-chloromercuribenzoic acid; *p*-chloromercuriphenyl sulfonic acid (n=5)

secretion was inhibited by mercurials.

DISCUSSION

The most important finding in this experiment was the observation the blocking effect of reducing agents to mercury vapor poisoning. The most severe pathological

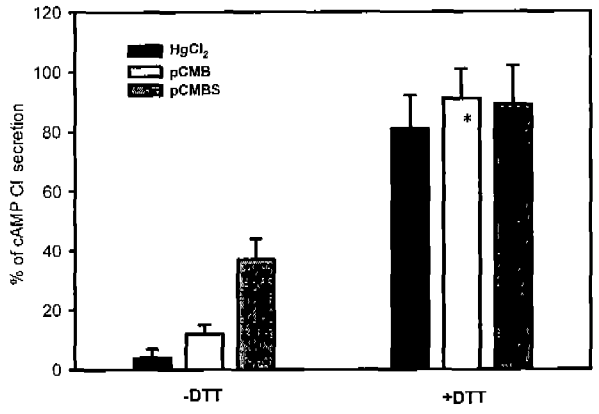

Fig. 3. The protective effect of dithiothreitol (DTT) on the inhibitory effects of mercurials.
The inhibitory effect of mercurials (Hg^{2+}, PCMB > 90%, PCMBS>80%) on the cAMP mediated Cl- secretion was restored after application of DTT (0.5 mM) (n=5)
**, P<0.01

change produced by mercury vapor (Hg0) and its dramatic protection by inhaled DTT was observed in the lung tissue in this experiment. The toxic effect of inhaled mercury to the kidney has been reported elsewhere, and it is not surprising that inhaled DTT protects renal pathological change as Hg^{2+} oxidized from absorbed Hg0 in the inside of respiratory cells eventually accumulates in the kidney. Based on our histopathological finding, we speculate that the direct cause of death by mercury vapor poisoning is ARF via an oxidized form (Hg^{2+}) converted from mercury vapor (Hg0).

As an oxidative damage to respiratory tract lining fluids (RTLF) by ozone (O_3) has been reported (24), and Silva *et al.* (25) reported that Hg^{2+} inhibited chloride secretion in the shark rectal gland, we inquired about the possible action of extracellular luminal Hg^{2+}, PCMB or PCMBS on the chloride secretion in RTLF of isolated airway epithelial monolayer. All these organic mercurials inhibited cAMP mediated chloride secretion in a dose dependent manner and the inhibitory effect was protected by DTT. We cannot conclude currently whether the action of organic mercurials in inducing ARF is intracellulary or extracellularly. As little as 10 nM apical Hg^{2+} inhibited cAMP mediated chloride secretion, which was completely reversed by the reducing agent in this experiment. Therefore, we speculate a small amount of oxidized form (Hg^{2+}) of inhaled mercury vapor (Hg0) might affect RTLF via a certain mechanism in respiratory epithelia.

Sulfhydryl groups (-SH) of cysteine residues of proteins are the most reactive of all amino acid side chains under physiological conditions. The cysteine residues that are adjacent in the three-dimensional structure of a protein can form a disulfide bridge. Yan & Maloney (26) reported that dramatic changes in the function of membrane proteins such as ion channel or carrier induced by the modification of cysteine residue.

PCMBS shares with PCMB a chemical specificity for the reaction with cysteine, but in PCBMS the weakly acidic carboxyl function of PCMB (pKa=4) is replaced by a strongly acidic sulfonic acid group (pKa=1.5), giving a water-soluble agent that is largely membrane impermeant and that should attack only those cysteines exposed to the external side. Based on the effect of PCMBS, we speculate that the target cysteine residues of Hg^{2+} might be present on the luminal side of respiratory epithelia.

Regardless of the target protein(s) of Hg^{2+} poisoning responsible for ARF, the protective effect of reducing agents of *in vivo* and *in vitro* study indicates that the administration of an aerosolized reducing agent can be an important strategy for an emergency manipulation of ARF patients suffering from mercury vapor poisoning. The results also indicate that dental amalgam should be used very carefully for those patients who have respiratory problems such as asthma.

ACKNOWLEDGMENT

The authors wish to acknowledge the financial support of Dong-A University made in the program year of 1997.

Thanks are due to professor Michael Duffy for proof-

reading the manuscript.

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(Received April 19, 2001; Accepted May 25, 2001)

초록 : 수은에 의한 급성호흡손상시 산화물질의 억제효과

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공기흡입을 통한 수은 중독시 치명적인 급성호흡중독을 유발하는 것으로 알려져 있다. 본 연구는 공기흡입에 의한 급성 호흡 중독의 기전을 밝히고자 하였다. 수은 흡입에 노출된 쥐의 60%가 72시간 이내에 사망하였지만 수은과 DTT 흡입을 함께 시켰을 경우 모든 쥐가 생존하였다. 조직학적 관찰은 수은 흡입이 직접적 사인임을 확인 시켜주었고 DTT 흡입 쥐의 조직은 현저히 보존되는 것을 관찰하였다. cAMP에 의한 Cl- 분비는 점막층의 수은염에 의해 억제되었으나 혈장측막 투여시 영향을 미치지 않았다. 수은에 의해 억제되는 Cl- 분비는 점막층에 DTT 투여시 완전히 회복되었다. 이와 같은 결과는 흡입한 수은의 산화형이 급성수은 중독에 기여함을 보여주며 공기흡입을 통한 급성수은 중독 환자를 다룰 수 있는 중요한 정보를 제공한다.